# Neurochemical Involvement in the Behavioral Effects of Brain Damage

Septal lesions differentially affect the performance of rats in a variety of aversive learning situations (see Caplan, 1973; Lubar, 1973; Defrance, 1976, for reviews). Many studies, for example, have demonstrated that septally-lesioned animals are deficient in passive avoidance learning (see Fried, 1972). Likewise, septal lesions severely disrupt the performance of rats in leverpress shock escape tasks (Gotsick, Osborne, Allen, & Hines, 1971). In contrast, septal lesions facilitate the acquisition of 2-way active avoidance and rats with septal lesions perform more efficiently than normal rats on Sidman avoidance tasks (Morgan & Mitchell, 1969). While several explanations have been proposed to explain these apparently descrepant results (see Caplan, 1973; Fried, 1972; Lubar, 1973), the most parsimonious explanation appears to be that septal lesions reduce freezing responses in aversive situations (Blatt, 1976; Mattingly, Osborne & Gotsick, 1979).

Following spetal lesions there is a significant reduction in the levels of several forebrain neurotransmitters including serotonin, acetylcholine, and the catecholamines, dopamine, and norepinephrine (see Defrance, 1976). Recently it has been suggested that the behavioral changes following septal damage are a consequence of the lesion-induced reduction of brain serotonin. Supporting evidence for this view is as follows: (a) Animals treated with para-chlorophenylalanine (PCPA), a compound which inhibits the synthesis of serotonin and thereby depletes brain serotonin, are similar to septally-lesioned rats in some aversive learning tasks. For example, PCPA-treated rats, like septal-lesioned rats, are deficient in passive avoidance but are superior to normal rats in active avoidance (see Peters, Anisman, & Papas, 1978); and (b) the facilitation of active avoidance learning following septal lesions is reversed by the administration of 5-hydroxytryptophan (5-HTP), compound which increases brain serotonin levels (Smith, 1979).

Although serotonin does seem to be involved in the septal lesion-induced changes in avoidance learning, recent experiments in our laboratory indicate that a reduction in brain serotonin cannot explain all the behavioral effects of septal lesions in aversive learning situations. As examples, we have found that rats treated with PCPA are less active than normal rats during an aversive conditioned stimulus (CS) (Mattingly, Chandler, Applegate, & Brunelle, 1981), whereas septally-lesioned rats are more active than normal rats during an aversive CS (Mattingly et. al., 1979). Further, although septally-lesioned rats are deficient in leverpress shock escape learning (Gotsick et al., 1971), rats treated with PCPA learn to escape shock as quickly as control rats (Mattingly, Graham & Applegate, 1981). Moreover, the deficient shock escape performance of rats with septal lesions is not improved by the administration of 5-hydroxytryptophan (Mattingly, Gotsick, Applegate & Graham, 1982). It is evident from these results, therefore, that the reduction of brain serotonin consequent to septal damage is not responsible for all of the observed lesion-induced behavioral changes.

As mentioned, septal lesions produce a reduction in the levels of other neurotransmitters besides serotonin. It is possible, therefore, that some of the behavioral changes following septal damage are a result of these other neurochemical changes. The objective of the present research, therefore, was to study the involvement of other neurochemical systems, besides serotonergic, in the behavioral effects of septal lesions. Specifically, the present experiments focused on the possible role of acetylcholine and dopamine in the deficient lever-press shock escape performance of rats with septal lesions.

## Experiment 1

The purpose of Experiment 1 was to determine the effect of a drug-induced interference with central cholinergic functioning on the lever-press shock escape performance of normal rats. Therefore, groups of rats were injected with either saline (control) or the central cholinergic antagonist, scopolamine (.2, 1.0, or 5.0 mg/kg) and then tested on a shock escape task. In addition, another group of rats was injected with 5.0 mg/kg of methylscopolamine prior to testing. Methylscopolamine is a peripheral cholingeric antagonist and therefore the inclusion of this group allows for a determination of central-peripheral effects of scopolamine. If reduced brain acetylcholine is responsible for the behavioral effects of septal lesions, then rats treated with scopolamine, like septally lesioned rats, should be deficient in shock escape learning and should show other behavioral characteristics of rats with septal lesions.

### Method

#### Subjects

Fifty male Wistar albino rats were experimentally naive and approximately 90 days old at the beginning of testing. All rats were housed individually and maintained on ad lib food and water. A 12 hour light-dark cycle was held constant throughout the experiment.

#### Apparatus

Behavioral testing was conducted in two Grason-Stadler operant conditioning chambers (Model IIII) housed individually in sound attenuated research chests. These chambers had grid floors, and a house light (GE 1820) and response lever mounted on one wall. Grason-Stadler constant current shock generators (Model 700) equipped with grid scramblers were used to deliver footshock.

#### Design and Procedure

The rats were randomly assigned, in equal numbers, to five drug condition groups. Three groups were injected intraperitoneally (IP) with doses of .2, 1.0, or 5.0 mg/kg of scopolamine hydrobromide.

One group was injected with saline, and the final group was injected IP with a 5.0 mg/kg dose of methylscopolamine hydrobromide. All injections were given 30 min before behavioral testing. All doses were calculated as the active base of the drug and dissolved in isotonic saline just prior to administration. Also, all doses were administered in a volume of 1 ml/kg and treatment conditions were coded so that group assignments were unknown to the experimenter during injection and testing procedures. Following the injection all rats were returned to their home cage.

Shock escape training was initiated 30 minutes following the drug injection. In each test session, the rat was placed into one of the chambers and 90 sec later a 1.0 mA footshock was delivered to the grid floor. This shock continued for 1 min or until the rat pressed the lever. The shock trials were separated by 90 sec intervals and the test session consisted of 60 discrete shock escape trials. During the test session, response latencies to the nearest .001 sec were recorded. Also, the total number of leverpresses (Barpresses) and the total amount of time the lever was depressed (Bartime) during the session was recorded.

## Results and Discussion

Figure 1 presents the mean speed scores for the groups across 6 blocks of 10 shock escape trials. Speed scores were derived by adding the integer one to each latency and then taking the reciprocal (i.e., 1/Latency + 1). This transformation prevents very short or very long latencies scores from making a disproportionate contribution to the mean performance scores. The possible range of transformed scores is from zero to one, with larger numerical scores representing faster response speeds.

As may be seen in Figure 1, all groups displayed an increase in speed scores across blocks, but the saline and methylscopolamine groups responded more quickly than the scopolamine-injected groups. An analysis of variance performed in these data revealed a significant effect of blocks, $F(5,225)=37.86$, $p < .001$, and a significant drug treatment effect, $F(4,45) = 4.71$, $p < .01$. A

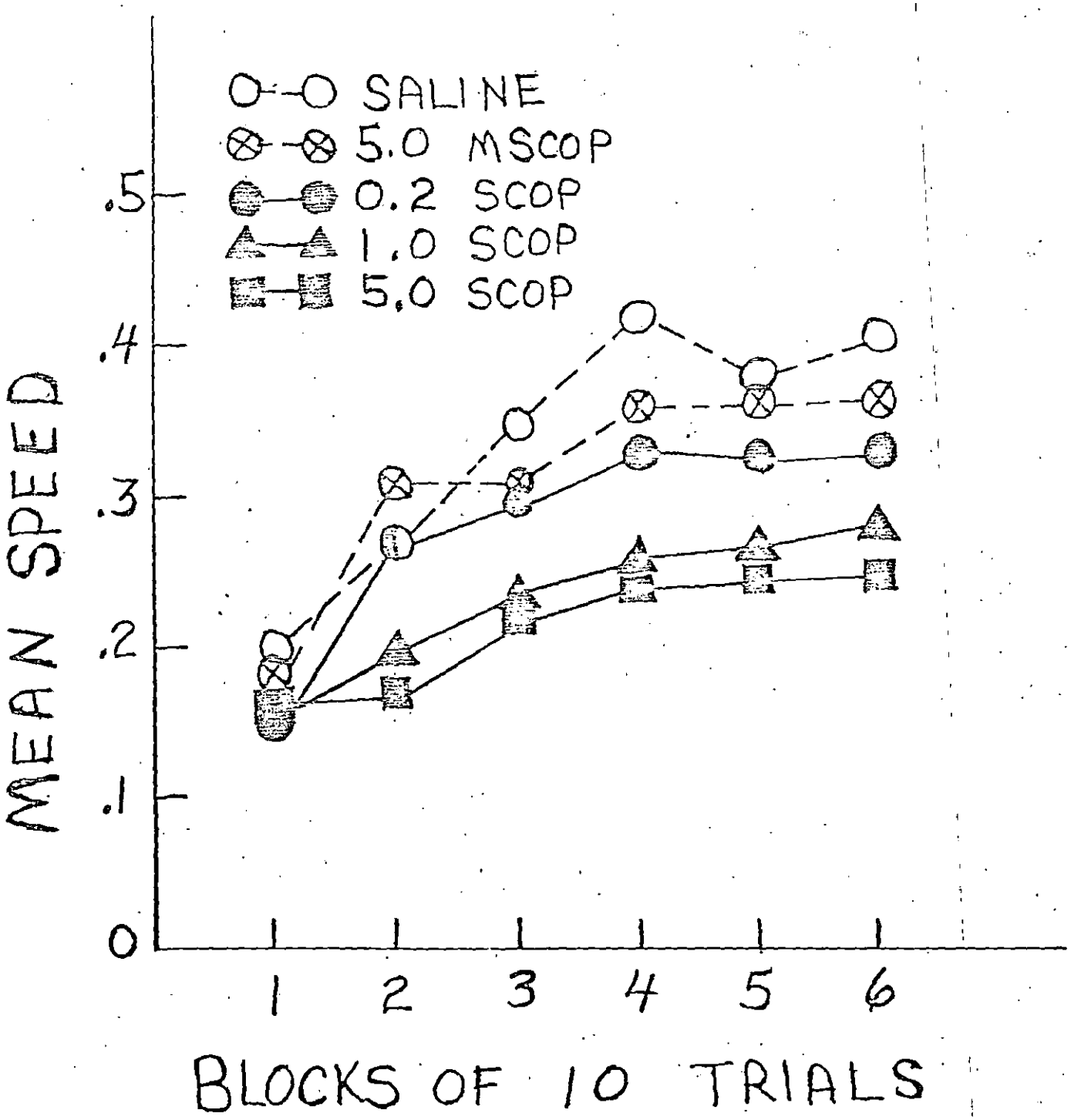


Figure 1. Mean speed scores across blocks of 10 trials for rats injected with 0.2; 1.0-, or 5.0 mg/kg scopolamine (Scop), saline, or 5.0 mg/kg of methylscopolamine (MSCOP).

Table 1

Summary of Mean Barpresses, Bartime and Bartime/Barpress (BT/BP) for the Five Drug Groups

| Drug | Barpress | Bartime | BT/BP |
|---|---|---|---|
| Saline | $191.2^{A}$ | $1258.2^{A}$ | $7.0^{A}$ |
| Methylscop | $111.2^{B}$ | $527.3^{B}$ | $4.9^{A}$ |
| Scop .2 mg/kg | $128.7^{B}$ | $95.7^{C}$ | $0.5^{B}$ |
| Scop 1.0 mg/kg | $124.6^{B}$ | $46.4^{C,D}$ | $0.3^{B}$ |
| Scop 5.0 mg/kg | $100.6^{B}$ | $27.1^{D}$ | $0.3^{B}$ |

Note: Means with different letter superscripts are significantly different as determined by Neuman-Keuls tests, $p < .05$

Neuman-Keuls post hoc analysis performed on the significant drug effect indicated that the saline-injected rats responded faster than the .2, 1.0, and 5.0 mg/kg scopolamine-injected rats, $p < .05$ in each case. Further, the methylscopolamine-injected rats responded significantly faster than the 1.0- and 5.0- mg/kg scopolamine-injected rats, $p < .05$ in each case. However, the saline group did not significantly differ from the methylscopolamine group in speed of responding, $p > .05$.

Table 1 presents the mean barpresses, bartime, and bartime per barpress for the five drug groups. An analysis of variance performed on each of these measures revealed a significant drug effect: barpress, $F(4.45) = 3.79$, $p < .01$; bartime, $F(4.45) = 12.35$, $p < .0001$; and bartime per barpress (BTBP), $F(4.45) = 10.85$, $p < .0001$. As may be seen in Table 1, the number of barpresses and amount of bartime was markedly decreased by scopolamine. More important, the decrease in bartime was not simply a function of decreased barpressing. Indeed, the saline-treated rats held the lever down an average of 7.0 sec for each barpress, whereas the rats injected with 5.0 mg/kg of scopolamine averaged less than 0.3 sec of bartime per barpress.

Previous studies (e.g., Gotsick et. al., 1971) have shown that normal rats learn to escape shock quickly by staying near the lever and holding it down during the intertrial intervals. Rats with septal lesions, however, have difficulty remaining near the lever during the intertrial intervals, and consequently, display very little bartime and much slower escape latencies than do normal rats. This pattern of slow escape responding and decreased bartime in rats with septal lesions is, of course, very similar to that observed in the present study with scopolamine-injected rats. Since septal lesions produce a significant reduction in acetylcholine activity, the results of the present experiment are consistent with the view that reduced brain acetylcholine may be responsible for the lesion-induced retardation of lever-press shock escape learning.

## Experiment II

The purpose of Experiment 2 was to determine whether dopaminergic mechanisms are involved in the lever-press shock escape performance of normal rats. Even though the results of Experiment 1 suggest the involvement of cholinergic mechanisms, because of the interaction among various transmitters in many central "pathways", many transmitters probably play some functional role in any complex response pattern of the organism. That is, although modification of the level of any one of the transmitter systems in the brain may have profound effects on behavior relatively independent of other transmitter systems, it is becoming more evident that many of these systems act in a reciprocally coordinated fashion (see Zolman, Mattingly, & Sahley, 1978).

In other areas of the brain (e.g., nigro-striatal), dopamine and acetylcholine have been found to function in precise balance such that decreasing the activity of one has more or less the same effect as increasing the other. With regard to the septal area of the limbic system, it is not known whether dopamine and acetylcholine function antagonistically. If so, then increasing dopaminergic activity should have an effect on shock escape learning similar to that of decreasing cholinergic activity with scopolamine. In Experiment 2, therefore, rats were injected with dopaminergic agonist, apomorphine, or saline and then tested on the same shock escape task used in Experiment 1.

### Method

#### Subjects, Apparatus, Design and Procedure

The subjects were forty male Wistar albino rats approximately 90 days old at the beginning of testing. The apparatus, rearing, and behavioral testing procedures were the same as in Experiment 1. The rats were randomly assigned, in equal numbers, to four drug condition groups. Three groups were injected IP with a single dose of either 0.5, 1.0, or 2.0 mg/kg of Apormorphine hydrochloride. The control group was injected with an equivalent volume of Saline. All injections were given 15 min before behavioral testing. All

doses were calculated as the active base of the drug and dissolved in isotonic saline just prior to administration. Also, all doses were administered in a volume of 1 ml/kg and treatment conditions were coded so that group assignments were unknown to the experimenter during both injection and testing procedures.

## Results and Discussion

The mean speed scores for the groups across the six blocks of 10 shock escape trials are plotted in Figure 2. Speed scores were derived as in Experiment 1. As may be seen in this Figure, the escape performance of the apormorphine injected rats was severely disrupted across the first three trial blocks, as compared to the saline-injected control rats. The apomorphine-injected rats, however, displayed a marked improvement in performance across the last three blocks and consequently, their performance differed little from the saline control rats on the final block of ten trials. An analysis of variance performed on these data revealed significant main effects for drug, $F$ (3,36)= 3.64, $p < .05$, for blocks, $F$ (5,180) = 60.40, $p < .0001$, and also a significant Drug x Block interaction, $F$ (15,180) = 3.52, $p < .0001$.

Like scopolamine - injected rats in Experiment 1, apomorphine-injected rats also exhibited significantly fewer barpresses than saline control rats, $F$ (3.36) = 3.34, $p < .05$. In contrast to scopolamine-injected rats, however, apomorphine-injected rats did not significantly differ from control rats in the amount of bartime, $F$ (3.36) = 1.68, $p > .05$. Moreover, apomorphine-injected rats displayed significantly more bartime per barpress than the saline control rats, $F$ (3.36) = 4.19, $p < .05$.

In summary, apomorphine produces only a temporary disruption of shock escape performance of normal rats, and this disruption does not appear to be due to the same behavioral mechanisms as that produced by either scopolamine or septal lesions. That is, both scopolamine and septal lesions produce a relatively permanent retardation of lever press shock escape learning and this disruption appears to be secondary to an inability of the rats to remain near

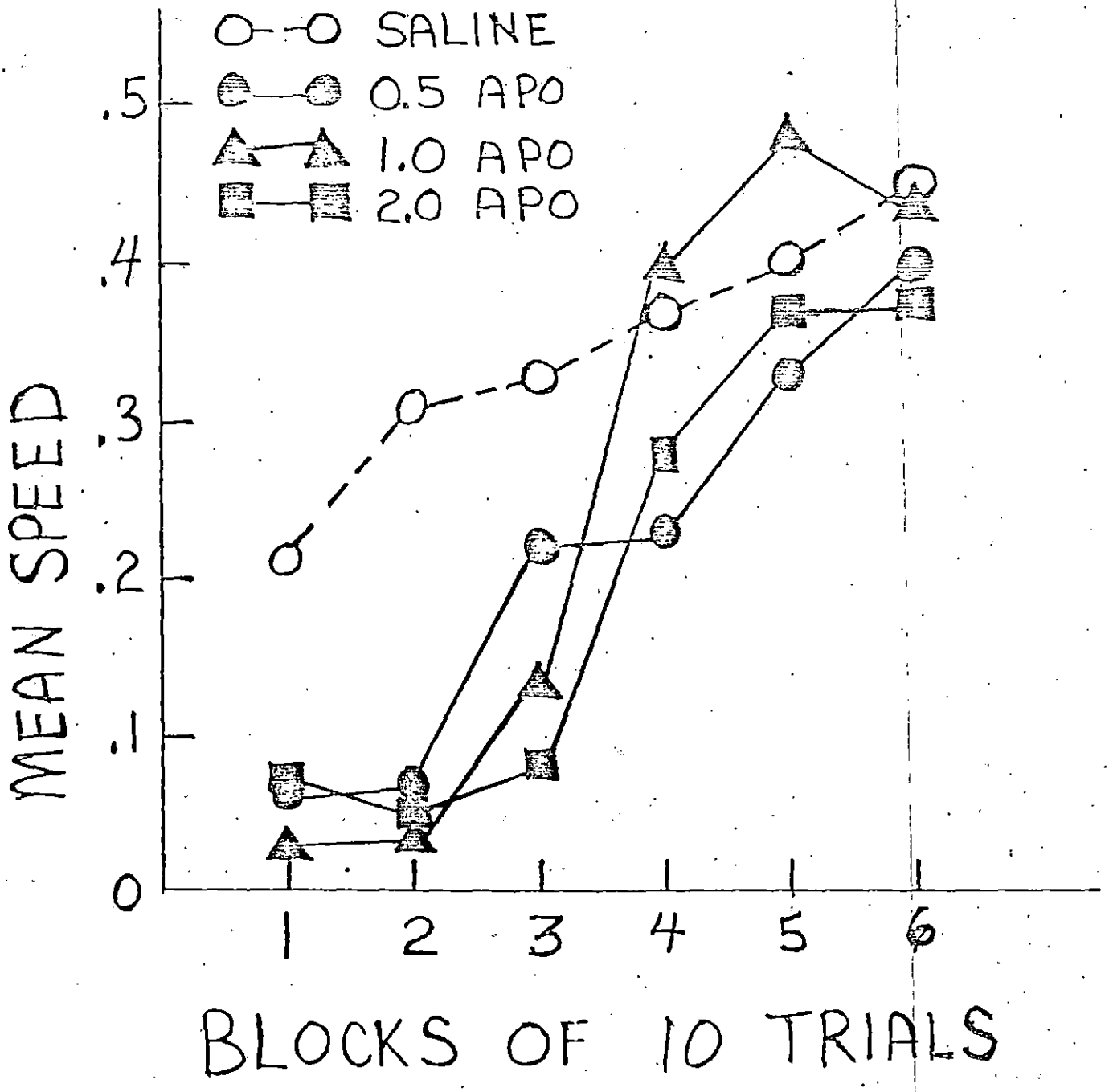


Figure 2. Mean speed scores across blocks of 10 trials for rats injected with 0.5-, 1.0-, or 2.0-mg/kg apomorphine (APO) or saline.

the lever and hold it down during the intertrial intervals. In contrast, apomorphine-injected rats did not differ from saline control rats in bartime and actually held the lever down more per leverpress than controls.

These differences in incidental behavior between apomorphine and scopolamine-injected rats suggest that acetylcholine and dopamine do not act in a reciprocally coordinated fashion with respect to shock escape learning. Moreover, these findings suggest that while reduced acetylcholine may be involved in the effects of septal lesions, reducing acetylcholine does not simply "unleash" an antagonistic dopaminergic system.

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